The Decomposition of 1-(β-D-Ribofuranosyl)-1,2-dihydropyrimidin-2-one (Zebularine) in Alkali: Mechanism and Products

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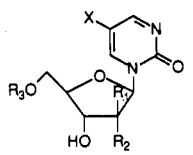
Received June 28, 1991 (Revised Manuscript Received October 1, 1991)

The mechanism of the base-catalyzed degradation of 1-(β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one (zebularine, **1a**) and closely related analogues was studied by NMR spectroscopy and GC–MS. Addition of sodium deuteroxide to a solution of **1a** in D_2O effected a rapid and irreversible reaction characterized by complete degradation of the heterocyclic pyrimidinone ring. ^1H NMR data suggested that **1a** was initially converted to the labile aldehyde **10**. This was later confirmed by similar degradation of the 5-fluoro analogue **1b** to the more stable aldehyde **9**. The alkaline degradation of **1a** reaches an end point after 4 h at room temperature with one identifiable product being the anomerized α-N1–O2 cyclic carbamate **6**. Compound **6** was formed by degradation of both **1a** and **1b**. The ara epimer **1c** formed the β-carbamate **8**, and the 5′-*O*-methyl derivative **1d** proceeded to the 5-*O*-methyl carbamate **7**. An inventory of the remaining atoms yields a formula which suggested the complementary component of the degradation to be an immediate precursor to 1,3-propane dialdehyde (malondialdehyde, MDA). Support for this proposal was evident in both the ^1H and ^{13}C NMR spectra of the basic reaction mixture which showed resonances that corresponded closely with those published for authentic MDA at pH 9.6. The presence of MDA was unequivocally proven by derivatization of the acidified degradation mixture with hydrazinobenzothiazole (HBT) to give the known adduct **11**. GC–MS analysis of the adduct obtained from HBT and the MDA formed during the decomposition reaction was identical to the adduct prepared from authentic MDA and HBT. Since the 5′-*O*-methyl derivative **1d** yielded the same type of products as those analogues with the 5′-hydroxyl free, it was concluded that the 5′-OH was not essential for alkaline lability. This contradicts the original literature assumption that some type of cyclization of the carbohydrate with the pyrimidinone system may be a first step in the mechanism. The data herein suggest that the base-catalyzed decomposition begins with the preferential attack at the 6-position of 2-pyrimidinone nucleosides. The discovery that a known mutagen (MDA) is a product in the degradation of **1a** suggests that a relationship could exist between the chemical susceptibility of **1a** and its unique biological activity.

Introduction

Zebularine [1-(β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one, **1a**] is a simple nucleoside which exhibits an unusual spectrum of biological activity. The synthesis of **1a** was first reported in 1961 by Funakoshi[1] and subsequently by several other groups.[2-4] Two of these laboratories[2,3] found that **1a** inhibited DNA synthesis in *E. coli*, and one traced this activity to a selective and irreversible inhibition of thymidylate synthetase via the 2′-deoxy-5′-monophosphate metabolite which formed intracellularly.[3]



1a: X=H, R_1=H, R_2=OH, R_3=H; Zebularine
1b: X=F, R_1=H, R_2=OH, R_3=H
1c: X=H, R_1=OH, R_2=H, R_3=H
1d: X=H, R_1=H, R_2=OH, R_3=CH_3

More importantly, zebularine was found to be a potent inhibitor of cytidine deaminase[5,6] (CDA) and also exhibited significant in vivo antineoplastic activity against several murine tumor types, particularly L1210 leukemia and B16 melanoma.[4] Similar activity with greatly increased potency was found for 5-fluorozebularine (**1b**).[4] The fact that **1a**, as opposed to other CDA inhibitors, is acid stable made it an attractive candidate for combination chemotherapy with antineoplastic drugs which are subject to enzymatic deamination, e.g., ara-C.[4] This high stability to acidic pH (<1.5) provided the basis for a preclinical *oral* combination study with ara-C.[4] The acid stability of **1a** stands in contrast to the prototype CDA inhibitor, tetrahydrouridine[7] (THU, **2**) which is known to rearrange to the inactive pyranose form at pH 1 with a half-life of ca. 30 min.[8]

The acid resistance of zebularine is in contraposition to its behavior in alkali. Oyen[2] has shown that a rapid and irreversible reaction occurred when **1a** was dissolved in 0.1 N NaOH solution characterized by a red shift in the parent UV maximum (from 303 to 315 nm). On neutralization a blue shift back to 277 nm was observed. Since the parent base pyrimidin-2-one and its 1-*N*-methyl derivative are resistant to alkali, it was assumed that the ribose portion of **1a** played an integral role in its sensitivity to alkali, possibly by some cyclization between the pyrimidinone and the carbohydrate.[2] Important studies by Undheim added credence to this argument when it was confirmed that strong nucleophiles add conjugately to the 4- and 6-positions of substituted 2-pyrimidinones.[9] Adduct formation was facilitated by electron-withdrawing groups at N1 and C5. Also, Wolfenden recently showed that the adduct of **1a** and water, catalyzed by CDA, is probably of the type **3**.[10] This covalent addition of water (or OH^-) to the 4-position of **1a** forms a transition-state analogue of hydrated amine **4**, the putative transition state adduct in the

(1) Funakoshi, R.; Irie, M.; Ukita, T. *Chem. Pharm. Bull.* **1961**, *9*, 406.
(2) Oyen, T. B. *Biochim. Biophys. Acta* **1969**, *86*, 237.
(3) Votruba, I.; Holy, A.; Wightman, R. H. *Biochim. Biophys. Acta* **1973**, *324*, 14 and references cited therein.
(4) Driscoll, J. S.; Marquez, V. E.; Plowman, J.; Kelley, J. A.; Barchi, J. J., Jr. Unpublished results.
(5) McCormack, J. J.; Marquez, V. E.; Liu, P. S.; Vistica, D. T.; Driscoll, J. S. *Biochem. Pharmacol.* **1980**, *29*, 830.
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(7) Camiener, G. W. *Biochem. Pharmacol.* **1967**, *17*, 1981.
(8) Kelley, J. A.; Driscoll, J. S.; McCormack, J. J.; Roth, J. S.; Marquez, V. E. *J. Med. Chem.* **1986**, *29*, 2351.
(9) Rise, F.; Undheim, K. *Acta Chem. Scand.* **1989**, *43*, 489.
(10) Frick, L.; Yang, C.; Marquez, V. E.; Wolfenden, R. *Biochemistry* **1989**, *28*, 943.

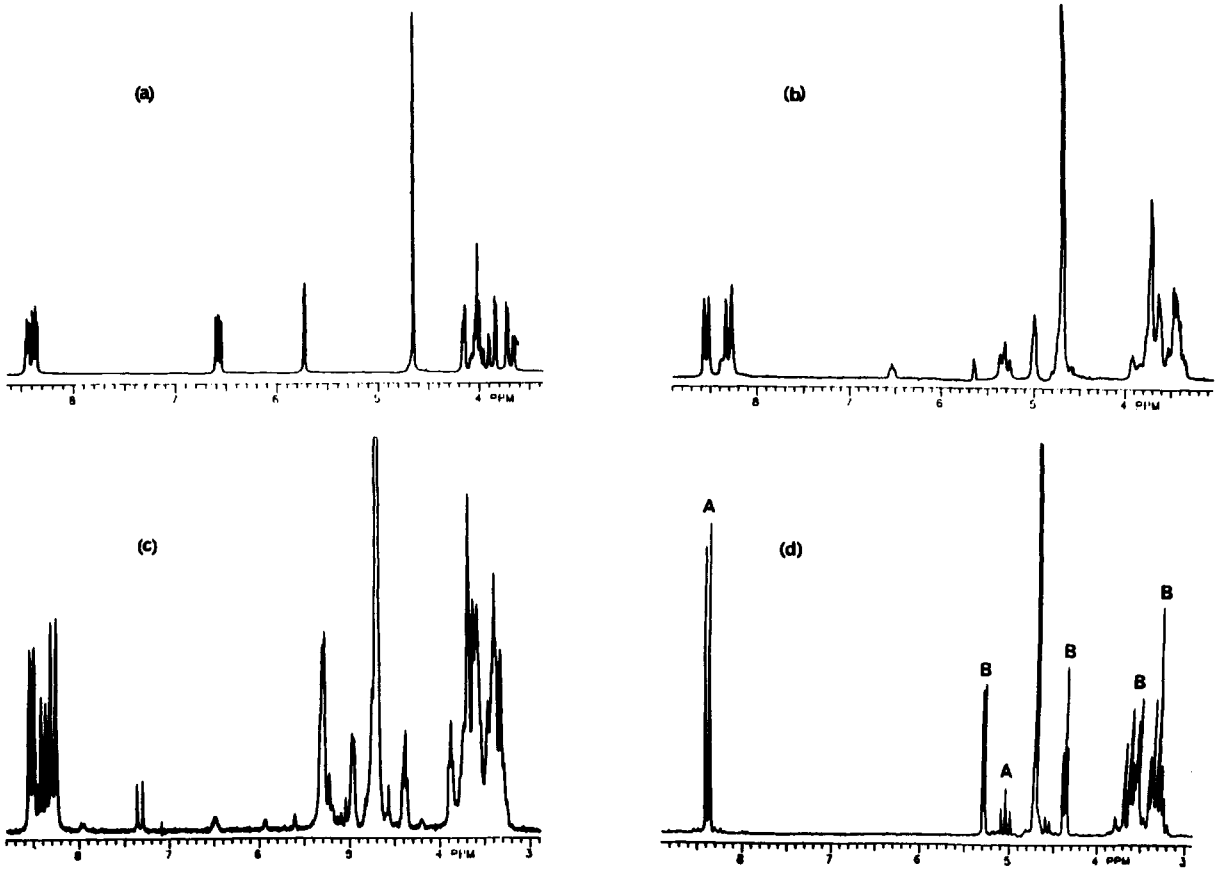
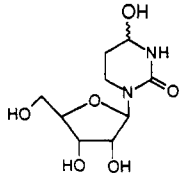
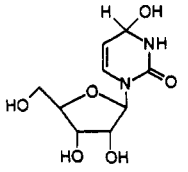


Figure 1. ^1H NMR spectra of compound **1a**: (a) in D_2O; (b) in D_2O with added NaOD at t = 5 min; (c) t = 60 min; and (d) t = 5 h.

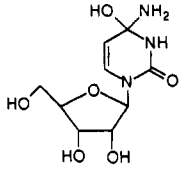
deamination of the natural substrate cytidine. These data, coupled with the intriguing spectrum of biological activity of **1a**, prompted us to undertake a more detailed investigation of zebularine in basic solution.



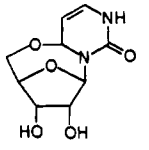
THU, 2



3



4



5

No reports have appeared concerning the aforementioned lability of **1a** since the work of Oyen.[2] In that publication, it was observed that although **1a** was sensitive to elevated pH, the aglycon, 2-oxopyrimidine and its N1-methyl derivative, were completely inert. At the time, it was reasoned that the carbohydrate portion of the molecule was probably an intermediary in the reaction, most likely via a cyclization of the ribose 5′-hydroxyl and the heterocyclic base. Circumstantial support for this postulate was offered by Liu et al.[11] who reported the characterization of the cyclization product **5** during the attempted preparation of the 2′,3′-isopropylidene derivative of **1a**.

In this report, we will show that a free hydroxyl group at the 5′-position of **1a** is *not* a requirement for the sensitivity of **1a** to alkali. In addition, the structures of the decomposition products have been rigorously characterized and some of the details of the mechanism leading to those products have been elucidated.

Results and Chemistry

Structure of Key Intermediates. Initially, the decomposition of **1a** and closely related analogues was studied by nuclear magnetic resonance (NMR) spectroscopy. Samples of the nucleosides were dissolved in D_2O at 25 °C in 5-mm NMR tubes, and a control spectrum was run (t = 0, Figure 1a). The pD of the D_2O solution was next brought to ca. 12 by addition of 50 μL of a 40% (w/w) solution of NaOD in D_2O, and a spectrum was recorded (t = 5 min, Figure 1b).[12] After such time, the ^1H NMR spectrum of **1a** experienced dramatic changes characterized by the appearance of two new doublets at δ 8.55 and 8.30 (J = 9.7 and 14.0 Hz, respectively) accompanied by almost complete suppression of H4, H5, and H6 of the starting nucleoside. After 1 h it was evident that zebularine was no longer present and the intensity of the aforementioned doublets was diminished at the expense of another new doublet at 8.39 ppm (J = 10.1 Hz). The carbohydrate protons experienced either complete reorganization (H1′ and H2′) or severe broadening and coalescence of their chemical shifts (H3′, H4′, H5′, and H5″).

(11) Liu, P. S.; Marquez, V. E.; Driscoll, J. S.; Fuller, R. W.; McCormack, J. J. *J. Med. Chem.* **1981**, *24*, 662.

(12) Spectra were run at 200 MHz at 22 °C; 32 transients, aquisition time, 2.549 s; 16K data points, 3000 Hz sweep width; 90° pulse at 15 μs; no temperature control was used.

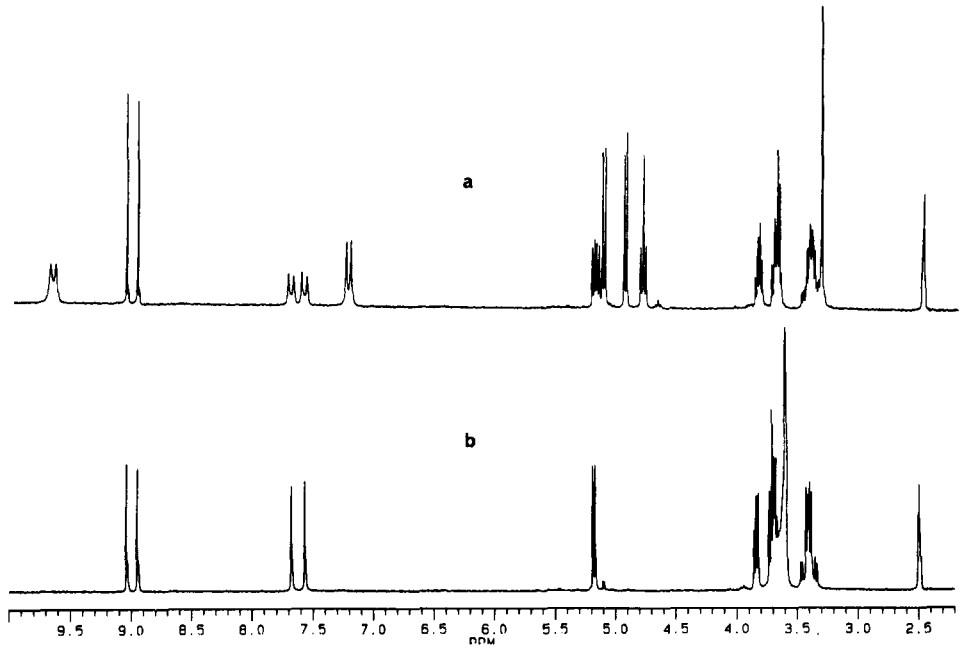
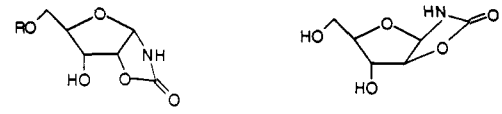


Figure 2. ^1H NMR spectra of compound 8: (a) in DMSO-d_6 and (b) in DMSO-d_6 with 1 drop of D_2O.

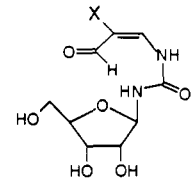
These conversions are shown in Figure 1c. During this period between 60 and 180 min several species were clearly visible but no additional information could be gleaned by NMR. After 5 h the spectra showed the formation of two distinct compounds (peaks labeled A and B, Figure 1d). The reaction seemed to reach an end point (i.e., no further changes in the NMR spectrum) after ca. 4 h at room temperature.

Compounds A and B were reasonably stable at high pH. Spectra recorded at 24, 48, and 168 h after treatment with NaOD showed no evidence of further decomposition. Hence, it was reasoned that the products might be isolable. To this end, **1a** was allowed to stand in an aqueous solution at pH 12 for 4 h whereupon the reaction was carefully neutralized with dilute HCl to pH 7.0.[13] During purification on C18 reversed-phase silica gel the compound corresponding to the peaks marked A was lost. The compound isolated gave rise to the peaks marked B in Figure 1d and appeared to contain the elements of the carbohydrate portion of zebularine after base treatment. Homonuclear decoupling experiments easily unraveled the spin systems in the ^1H NMR spectrum of B. The ^{13}C NMR revealed six carbon signals, five of which closely resembled those of a ribose ring. An additional quaternary signal was observed at 162.9 ppm. Fast-atom bombardment mass spectrometry (FABMS) suggested a molecular weight of 175 amu (MH^+ = 176) confirming the presence of nitrogen. These data allowed us to propose the cyclic 1,2-carbamate **6** as one final product in the decomposition of **1a**. It was evident that the ribofuranose ring remained more or less intact but the H1–H2 and H2–H3 coupling patterns suggested that an epimerization had occurred at C1. Interestingly, the 5-fluoro- and 5′-*O*-methylzebularine analogues (**1b** and **1d**) decomposed in a like manner under identical conditions to the products **6** and **7**. The 2′-ara derivative **1c** yielded the β carbamate **8** as its degradation product. The assumption by Oyen that the carbohydrate portion of **1a** may be involved in a cyclization with the 2-pyrimidone aglycon is, therefore, probably not correct based on the formation of **7** from **1d**. This does not, however, preclude the participation of the 5′-OH in the degradation of **1a**, **1b**, and **1c**, but suggests that a free OH at the 5′-position is not a requirement for their instability.

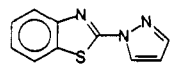


6 R = H
7 R = CH_3

8



9 X = F
10 X = H

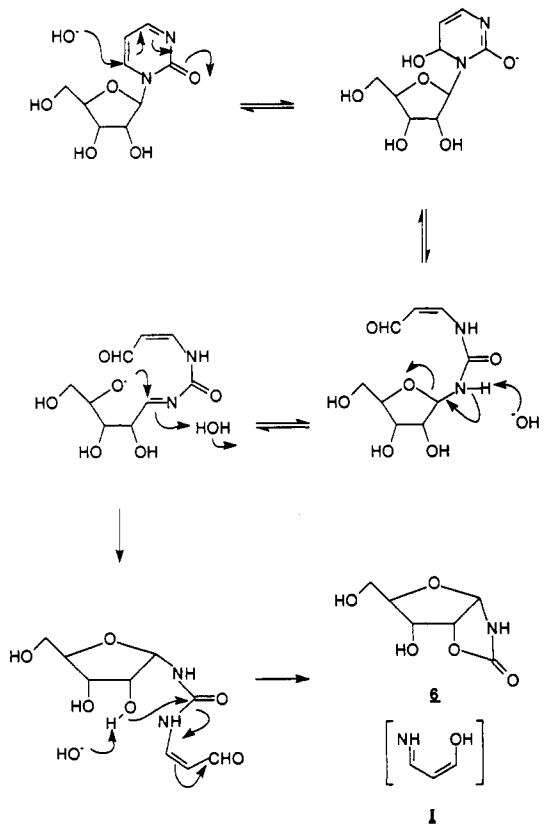


11

In an effort to characterize the intermediates in the decomposition reaction zebularine (**1a**) and 5-fluorozebularine (**1b**) were treated with base and the reaction was quenched by neutralization with dilute HCl at different time intervals. Since the NMR data showed that extensive transformation had taken place after 2 min, both **1a** and **1b** were reacted with aqueous NaOH (pH 12) for 2 min and the solutions carefully neutralized as before. Other than uncharacterized decomposition products (<-20%), the alkaline solution of **1a** yielded only starting nucleoside after purification. In contrast, the short-term decomposition of the 5-fluoro derivative **1b** was highly informative, yielding one stable product after HPLC purification. Selected NMR spectra of this compound are shown in Figure 2. As expected from similar experiments with **1a**, the carbohydrate portion of the molecule was virtually unchanged. When D_2O was added, the spectrum simplified to that shown in Figure 2b. It was clear that the two strongly coupled doublets in the low-field region of the spectrum were due to three-bond ^1H–^{19}F coupling. The proton-decoupled ^{13}C NMR spectrum displayed a doublet of 14.6 Hz for a carbon signal absorbing at 186.3 ppm. A Fourier-transformed infrared spectrum showed

(13) pH was monitored to three significant figures with a Beckman Φ 45 pH meter at 24.7 °C.

Scheme I



a strong band at 1720 cm^{-1}. These data were indicative that the aldehyde **9** formed after brief treatment of **1b** with base. Further studies with the nonfluorinated compound **1a** at longer reaction times (i.e., 1 h) yielded a mixture of compounds, which, by NMR, seemed to include the corresponding aldehyde **10** from zebularine. Unfortunately, this aldehyde was much more reactive than **9** and, after neutralization, a significant amount recyclized back to the parent nucleoside.

In an attempt to generate a more isolable derivative of aldehyde **10**, a 1-h degradation experiment was performed with **1a** as described above. After neutralization and lyophilization, the residue was acetylated at 0 °C. The major product of this reaction was 2′,3′,5′-zebularine triacetate which was identical with the product obtained from peracetylation of **1a** with Ac_2O/pyr. A second compound which coeluted with the triacetate was tentatively assigned as the peracetate of the aldehyde **10** by homonuclear decoupling experiments performed on a mixture of this peracetate and zebularine triacetate. The chemical shifts and coupling constants of the observed spin systems seem to match well with the proposed α,β-unsaturated aldehyde system of **10**. A plausible pathway for the formation of **6** from **1a**, based on these data, is outlined in Scheme I.

The Remaining Atoms. With physical proof that compound **6** was produced from **1a** in the presence of water and hydroxide ion, the remaining task was ascertaining the fate of the "upper half" of the pyrimidinone ring. If the mechanism in Scheme I is correct, atoms 3–6 would yield a compound of general structure I. In aqueous solution, I should hydrolyze and tautomerize to 1,3-propanedialdehyde more commonly referred to as malondialdehyde (MDA).

It was stated earlier that the peaks marked A in Figure 1 were lost upon neutralization and purification. These two multiplets represented a simple coupled system as determined by homonuclear decoupling experiments. It was found that the triplet at 5.1 ppm was not coupled anywhere else in the spectrum. Also, the intensity of the doublet at 8.4 ppm (J = 10.1 Hz) suggested at least two magnetically equivalent protons absorbed at this chemical shift. These data were puzzling until a recent report by Bertz[14] which tabulated the ^1H NMR spectra of MDA at various pH values. When an aqueous solution of MDA (prepared by literature procedures[15]) was adjusted to pH 9.6 with NaOH, the two signals observed were a doublet at 8.64 ppm (two protons) and a triplet at 5.30 ppm with a coupling constant of 10.1 Hz. The ^{13}C NMR spectrum of a mixture of **1a** and NaOD (after stirring at rt for 4 h) revealed a set of signals which also correlated well with those observed by Bertz for MDA (Experimental Section of ref 14). MDA may exists in several different forms in solution; in water it has been reported[15b] to be in the trans enol conformation (eq 1). This structure undeniably explains the NMR data obtained on the decomposition mixture at t = 4 h.

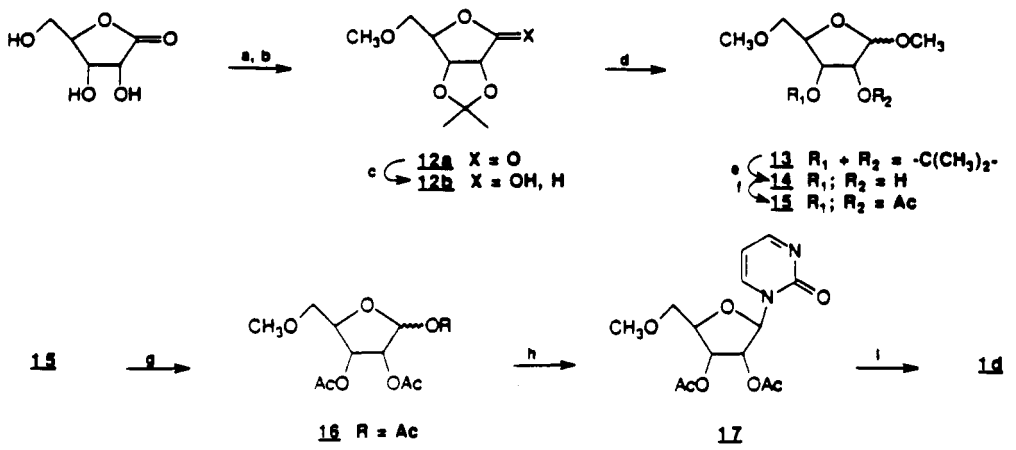
(1)

If MDA is the byproduct of the decomposition of **1a**, it is clear why this labile electrophile was not isolated under the workup conditions mentioned above. It was reasoned that formation of a derivative of MDA at a judicious stage in the degradation sequence would facilitate isolation and unequivocally prove its liberation from **1a**. To this end we were prompted by a recent report[16] which documented the characterization of a pyrazole adduct of MDA and hydrazinobenzothiazole (HBT). In that work, when a solution of MDA (generated by the hydrolysis of 1,1,3,3-tetramethoxypropane, TMP, with 0.1 N HCl) and HBT in 0.1 N HCl was heated to 100 °C for 1 h the cyclic pyrazole derivative **11** was isolated as a crystalline solid. In our experimental system, one needs to convert the purported MDA, which exists as sodio-MDA at pH 12, back to the free enol by acidification, and while at low pH, form the HBT-MDA adduct. This presupposes the absence of any acid labile products in the mixture. Since it was observed that the only products at t = 4 h seemed to be the relatively unreactive compound **6** and MDA, this was not a major concern.

The HBT–MDA adduct was first prepared as a standard according to the published procedure.[16] Meanwhile, in a separate experiment, compound **1a** (170 mg) was decomposed at pH 12 for 4 h. The decomposition reaction was acidified to pH 4, and 2 equiv (based on the amount of **1a**) of HBT was added to the solution. Since HBT was not very soluble in this solution, the pH was lowered to ca. 3 and the reaction was heated as was done for the formation of the standard adduct. After 10 min, half of the mixture was separated and analyzed by GC–MS. The major peak in the total ion chromatogram (TIC) from the HBT reaction with the degradation products of **1a** was identical to that obtained by reacting HBT with MDA in both column retention time and molecular mass. Of the three remaining peaks, one was determined to be unreacted hydrazine (HBT) and another was benzothiazole according to MS[17] (m/e 165 and 135, respectively). The remaining peak (m/e 150) conceivably could be benzothiazolamine

(14) Bertz, S. H.; Dabbagh, G. *J. Org. Chem.* **1990**, *55*, 5161.
(15) (a) Protopopova, T. V.; Skoldinov, A. P. *Zh. Obshch. Khim.* (*Engl. Ed.*) **1958**, *28*, 241. (b) George, W. O.; Mansell, V. G. *J. Chem. Soc. B* **1968**, 132.
(16) Beljean-Leymarie, M.; Bruna, E. *Anal. Biochem.* **1988**, *173*, 174.
(17) Heller, S. R.; Milne, G. W. A. *EPA/NIH Mass Spectral Data Base*; U.S. Government Printing Office: Washington, DC, 1978; Vol I.

Scheme II[a]



[a] Key: (a) acetone, H^+; (b) MeI, Ag_2O (compound **12a**, see ref 24); (c) DIBAL, −78 °C; (d) TsOH, MeOH; (e) 2 N HCl, MeOH; (f) Ac_2O, TEA, DMAP; (g) AcOH, Ac_2O, H_2SO_4; (h) TMS-pyrimidin-2-one, TMS-Tf, CH_2Cl_2; (i) NH_3, MeOH.

resulting from N–N cleavage.[17] When the remaining volume of the reaction mixture was heated to 100 °C for 1 h, a TIC consisting of essentially pure HBT–MDA adduct was obtained by GC–MS. Thus, conclusive evidence for the formation of MDA from the degradation of **1a** in base had been established.

Synthesis of Compounds 1c and 1d. The ara derivative **1c** and 5′-*O*-methylzebularine (**1d**) were previously synthesized by Holy et al.[6] as structural variants of **1a** for cytidine deaminase structure–activity studies. Compounds **1c**[18a] and **1d**[18b] were also prepared in this laboratory.

Discussion

Scheme I depicts the proposed mechanism for the base-catalyzed decomposition of **1a** which closely matches the empirical results. The formation of 5-*O*-methyl carbamate **7** instructed us to begin with hydroxide ion attack, in a conjugate fashion, on the heterocyclic ring as a first step. Simple tautomerization yields the ring-opened aldehyde, which, as was shown in Eq 1, is in dynamic equilibrium with the dehydrated closed form of the aglycone. Rationalization for an anomerization step is supported by the following: (1) removal of a proton adjacent to the electron deficient C1′ center from the anomeric amido nitrogen is energetically favored by extending the conjugated system of the intermediate shown; (2) rotation about the C2′–C3′ bond to facilitate reclosure to an α-ribosyl derivative creates a favorable geometry for reorganization of the reactive side chain with the now proximate 2′-hydroxy group. This also prompts the construction of a thermodynamically stable cis-fused [5.5.0] ring system; hence, the formation of the β-cis-fused carbamate from the decomposition of the arabinofuranosyl analogue **1c**. The electrophilic urea carbonyl is now primed for carbamate formation with concomitant release of a three-carbon electron acceptor which accounts for the elements of MDA. This mechanism also accounts for the formation of the arabino derivative **8** from the degradation of **1c**. During breakdown of the heterocyclic ring the elements of isocyanate were presumably captured by the 2′-hydroxyl group which by being in the ara configuration facilitated carbamate formation prior to epimerization. One should note that this proposed transformation of the pyrimidinone ring of **1a** with base is formally the reverse of the synthesis of pyrimidin-2-one which has been prepared by condensation of MDA with urea (N1 of the pyrimidinone bearing H in place of ribosyl).[19]

MDA is produced as a primary product of lipid peroxidation in animal tissue.[20] This highly reactive aldehyde is known to covalently bind or cross-link biological macromolecules.[21] Studies have shown that MDA reacts with amino acids[22] and nucleoside bases[21,23] to form some unusual adducts under rather tolerable conditions (pH 4–7, 25 °C). This behavior may be the basis for its documented carcinogenic and toxic effects. It seemed reasonable to us that, based on the products generated in the decomposition described above, MDA, or an isoreactive equivalent of it, may be responsible, in part, for some of the biological properties of zebularine. It is highly unlikely that zebularine would encounter such strongly alkaline conditions as those used in this study. Notwithstanding, the 2-oxopyrimidine ring could be "activated" by other means. The aforementioned work of Undheim[9] proved that thio nucleophiles readily add to this ring system in Michael-type fashion. He also proved the possibility of potentiating the electrophilic character of the system by a judicious substitution pattern at either positions 4 or 5 of the heterocycle. Using the information described here and elsewhere[9,21,23] it may be possible to design a "fine tuned" derivative of **1a** which would utilize endogenous nucleophiles (i.e., thiols of sulfur containing proteins, N7 or N3 of purine nucleotides, etc.) to facilitate the release of MDA. A reasonable starting point for this type of design may be with compound **1b**, whose potency greatly exceeds that of **1a**.

On the basis of our results, it is interesting to note that the ribose ring of **1a** appears to be acting as an electron-withdrawing group[25] since the susceptibility of the heterocycle to alkali is negligible when the sugar is absent. It

(18) (a) Barchi, J. J., Jr.; Haces, A.; Marquez, V. E. Unpublished results. (b) The 5-*O*-methyl derivative was prepared by a slightly improved route; see Scheme II.

(19) Hamberg, M.; Niehaus, W. G. and Samuelsson, B. *Anal. Biochem.* **1968**, *22*, 145.
(20) Pryor, W. A. *Free Radicals in Biology*; Academic Press: New York, **1976**; Vols I and II.
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(22) Nair, V.; Vietti, D. E.; Cooper, C. S. *J. Am. Chem. Soc.* **1981**, *103*, 3030.
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seems that the hemiaminal C1 carbon (formally an aldehyde) imparts enough electrophilic character to position 6 of the ring to allow decomposition to take place. Studies directed toward the quantum mechanical reasons for this behavior, as well as toward the preparation and decomposition of other derivatives of **1a**, are currently in progress.

Experimental Section

Infrared spectra were recorded in the FT mode. Optical rotations were recorded at the sodium D line. Proton and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively. Chemical shifts are referenced against the solvent in which the samples were run. Electron impact mass spectra were obtained on a mass selective detector (MSD), operating with a source temperature of 250 °C and ionizing energy of 70 eV. Scans were acquired over a mass range of 50–500 amu at a rate of 1.07 scan/s. The malondialdehyde derivative was introduced into the MSD by gas chromatograph using a J&W (Folsom, Ca) DB-1 capillary column (250 μm i.d. × 15 M). The GC was programmed from 150 to 250 °C at a rate of 25 °C/min. Under these conditions the malondialdehyde derivative eluted in 6.32 min. Positive-ion fast-atom bombardment mass spectra (FABMS) were obtained at an accelerating voltage of 6 kV and a resolution of 2000. Glycerol was used as the sample matrix and ionization was effected by a beam of xenon atoms. During mass spectral aquisition the instrument was scanned over the mass range 50–800 at 10 s/dec. Analytical TLC analysis was performed on silica gel (250 μm). Column chromatography was accomplished with Kieselgel 60 (mesh size 230–400). Reversed-phase purification was performed either on C18 disposable extraction columns or by HPLC on a Beckman Ultrashere C18, 25 cm column (5 μm). Variable-wavelength UV detection was supplied during chromatography. Moisture-sensitive reactions were run under argon in flasks previously dried at 110 °C. Dry solvents were from Sure Seal bottles purchased from Aldrich Chemical Co.

Compounds **1a** and **1b** were obtained from the Drug Synthesis and Chemistry Branch, NCI, and compound **1c** was synthesized in the Laboratory of Medicinal Chemistry, NCI, NIH.

General Procedure for Base-Catalyzed Degradation Studies. Degradations of the various zebularine derivatives were carried out in 20-mL disposable vials or in 5-mm high-resolution NMR tubes and monitored by ^1H and ^{13}C NMR analysis. For the preparative experiments, 50–200 mg of the derivative was dissolved with stirring in 3–7 mL of H_2O and the pH was adjusted to 12 with concentrated NaOH. After an allotted time the reaction mixture was neutralized with HCl to a pH of 7.0 + 0.1 unit. This was lyophilized and desalted by passage through a C18 reversed-phase extraction cartridge (Baker) by elution with water. Final purification, where applicable, was effected by HPLC on a Beckman Ultrasphere 5-μm ODS column using mixtures of acetonitrile/water as the mobile phase. For NMR scale reactions, each sample consisted of 18–25 mg of derivative in 0.5 mL of D_2O. After a control spectrum was run, two drops (0.05 mL) of a 40% solution of NaOD (w/w in D_2O) was added, and the ^1H NMR spectrum was recorded at various time intervals. For most degradations the reaction was essentially complete after 4 h.

Isolation of 1-Amino-(1,2-carbamoyl)-α-D-ribofuranose (6). The benchtop procedure was followed with 100 mg of zebularine (**1a**) in 5 mL of water. After NaOH treatment, neutralization, and desalting, the residue was purified by reversed-phase HPLC on a Beckman column (25 cm) employing 6% CH_3CN/H_2O as the eluant. The cyclic carbamate **6** was isolated in ca. 30% yield and characterized as described below: ^1H NMR (D_2O) δ 5.66 (d, J = 5.4 Hz, H1), 4.99 (t, J = 5.4 Hz, H2), 4.03 (dd, J = 5.5 and 9.3 Hz, H3), 3.81 (dd, J = 2.0 and 12.4, H5), 3.75 (m, H4), 3.61 (dd, J = 4.5 and 12.4 Hz, H5′); ^{13}C NMR (D_2O) 162.98, 87.97, 82.41, 80.65, 72.45, 62.26; FABMS 176 m/e (MH^+). Anal. Calcd for $C_6H_9NO_5 \cdot 0.33H_2O$: C, 39.78; H, 5.34; N, 7.73. Found: C, 39.99; H, 5.12; N, 7.44.

1-Amino-5-*O*-methyl-(1,2-carbamoyl)-α-D-ribofuranose (7). The 5-*O*-methyl carbamate was isolated in a similar manner to compound **6**. The nucleoside **1d** was allowed to react with NaOH for 4 h and the pH was lowered to 7.0. The solution was lyophilized and the residue filtered through a C_{18} extraction column with water as the eluant. An analytically pure sample was obtained by reversed-phase flash chromatography on Bakerbond C18 silica (40-μm LC packing) using water as the eluant: ^1H NMR (D_2O) δ 5.63 (d, J = 5.4 Hz, H1), 4.96 (t, J = 5.4 Hz, H2), 3.99 (dd, J = 5.4 and 9.6 Hz, H3), 3.80 (m, H4), 3.66 (dd, J = 2.1 and 11.6 Hz, H5), 3.50 (dd, J = 5.6 and 11.6 Hz, H5′); ^{13}C NMR (DMSO-d_6) δ 158.00, 84.65, 78.16, 76.85, 71.16, 70.77, 58.52; FABMS m/e 190 (MH^+). Anal. Calcd for $C_7H_{11}NO_5$: C, 44.44; H, 5.82; N, 7.41. Found: C, 44.35; H, 5.86; N, 7.40.

1-Amino-(1,2-carbamoyl)-β-D-arabinofuranose (8). The ara epimer **1c** was treated as **1a** above and the reaction allowed to proceed at room temperature for 4 h. After neutralization and desalting on a C18 extraction column the carbamate **8** was isolated as a crude oil whose NMR spectrum differed from **6** only in the coupling constants of the H1, H2, and H3 protons: ^1H NMR (D_2O) δ 5.73 (d, J = 5.8 Hz, H1), 4.90 (dd, J = 1.8 and 5.8 Hz, H2), 4.25 (dd, J = 3.8 and 1.8 Hz, H3); high-resolution MS calcd 175.0559, found 176.0561. Due to the small amount of material on hand and the low yield of the degradation, this compound was not analyzed further.

1-[*N*′-(2-Fluoro-3-oxopropenyl)ureido]-β-D-ribofuranose (9). The general procedure was employed where a solution of **1b** was kept at pH 12 for 2 min. After neutralization and purification by C18 extraction cartridge eluting first with water and then methanol, the fluoro aldehyde **9** was obtained as an oil from the organic washings. This could be further purified by reversed-phase HPLC to give fairly pure **9**: IR (NaCl) 1720.9, 1643.8, 1549.0, 1352.9, 1209.8, 1034.7 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.70 (d, J = 11.5 Hz, exchangeable, N3-H), 9.04 (d, J = 22.6 Hz, C*H*O), 7.65 (dd, J = 11.7 and 27.5 Hz, H6), 7.51 (d, J = 9.5 Hz, exchangeable N1-H), 5.20 (dd, J = 5.2 and 9.5 Hz, H1′), 5.13 (d, J = 6.0 Hz, exchangeable OH), 4.94 (d, J = 5.3 Hz, exchangeable OH), 4.80 (t, J = 5.5 Hz, exchangeable 5′-OH), 3.85 (m, H2′), 3.70 (m, H3′ and H4′), 3.30–3.50 (m, H5′ and H5″); ^{13}C NMR (DMSO-d_6) δ 186.22 ($^2J_{C,F}$ = 14.6 Hz, *C*HO), 157.36, 148.94 ($^1J_{C,F}$ = 242.4 Hz, C5), 134.50, 89.78, 88.87, 79.63, 75.60, 66.96. Anal. Calcd for $C_9H_{13}N_2O_6F \cdot 0.5\ H_2O$: C, 39.56; H, 5.12; N, 10.25; F, 6.96. Found: C, 39.19; H, 5.05; N, 10.04; F, 7.07.

HBT–MDA Adduct (11). The adduct was prepared as a standard from HBT and 1,1,3,3-tetraethoxypropane in 0.1 N HCl at 100 °C. At the same time 170 mg (0.75 mmol) of **1a** in 5 mL of water was decomposed in base for 4 h. The alkaline reaction mixture was carefully neutralized to pH 3.83 with dilute HCl (total volume ca. 10 mL), and 246 mg (1.5 mmol) of HBT was added immediately. After stirring at 40 °C for 5 min the pH of the solution was lowered to ca. 3, the mixture was heated to 100 °C for an additional 10 min, half of the volume of the reaction mixture was removed and extracted with CH_2Cl_2 (3×). The combined extracts were washed with water, saturated sodium bicarbonate solution, and brine, dried (sodium sulfate), and concentrated. GC–MS analysis of a small amount of this crude product exhibited a major peak with characteristics identical to the standard adduct along with three additional peaks. When the pH of the remaining solution was lowered still (ca. 2) and heated to 100 °C for another 1 h and the mixture extracted as above, the HBT–MDA adduct was essentially the sole product by GC. Adduct retention time: 6.32 min at 215 °C oven temperature. m/e = 201 amu.